

Principle of Affinity Chromatography

The principle of affinity chromatography is based on the specific and reversible interaction between a target molecule and a ligand immobilized on a solid support. Affinity chromatography is widely used for the purification and isolation of biomolecules, such as proteins, enzymes, antibodies, and nucleic acids, based on their specific binding properties. Here is an outline of the principle:

Ligand immobilization: A solid support, such as a resin or a column matrix, is functionalized with a ligand that specifically interacts with the target molecule of interest. The ligand can be a small molecule, protein, antibody, or nucleic acid that recognizes and binds to the target molecule with high affinity and specificity.

Sample application: The sample containing the mixture of molecules, including the target molecule, is applied to the affinity column or resin. The target molecule selectively interacts and binds to the immobilized ligand, while other non-target molecules pass through or weakly bind to the support.

Washing: After sample application, the column or resin is washed with a buffer to remove unbound or weakly bound molecules. This step helps to minimize non-specific binding and remove contaminants.

Elution: The bound target molecule is then eluted from the affinity column or resin by introducing a specific elution buffer. The elution buffer disrupts the interaction between the target molecule and the immobilized ligand, allowing the target molecule to be collected in a purified form. Elution can be achieved by altering the pH, ionic strength, or using competitive binding agents that compete with the target molecule for binding to the immobilized ligand.

Elution profile and collection: During elution, the target molecule is detected and monitored using appropriate detectors, such as UV absorbance or fluorescence, depending on the nature of the molecule. The eluted fractions containing the purified target molecule are collected for further analysis or downstream applications.

Affinity chromatography provides high selectivity and specificity in the purification of target molecules, as the immobilized ligand specifically recognizes and binds to the target molecule. The

choice of the ligand is crucial and depends on the specific affinity interactions of the target molecule. Affinity chromatography is a powerful technique used in various fields, including protein purification, antibody purification, protein-protein interaction studies, drug discovery, and diagnostics.